Case Report

Epstein-Barr Virus Associated Lymphoepithelial Carcinoma of the Parotid Gland; A Clinicopathological Report of Three Cases

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Lymphoepithelial carcinoma is a relatively uncommon malignant tumor of the salivary gland demonstrating malignant epithelial cells with dense lymphoid stroma. The authors report three cases of lymphoepithelial carcinoma associated with Epstein-Barr virus of the right parotid gland with clinically presenting as painless, gradual enlargement of the preauricular mass. The histopathologic examination of the parotid gland is characterized by malignant epithelial cells with dense lymphoid stroma. Immunohistochemical stains show positive reactivity to cytokeratin and p53 in malignant epithelial cells. In situ hybridization of the Epstein-Barr virus-encoded Ribonucleic acid shows positivity in malignant epithelial cells. Clinical and pathologic features with relevant literatures are discussed. These are the first reported cases of primary parotid lymphoepithelial carcinoma associated with Epstein-Barr virus infection in Thailand and Southeast Asia.

Keywords: *Lymphoepithelial carcinoma, Undifferentiated carcinoma, Parotid gland, Epstein-Barr virus, In situ hybridization*

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Epstein-Barr Virus (EBV) is a member of the family Herpesviridae. EBV plays an important role on the pathogenesis of nasopharyngeal carcinoma, Burkitt's lymphoma, and Hodgkin's disease[1]. Lymphoepithelial carcinoma (LEC) is a relatively uncommon malignant salivary gland tumor demonstrating malignant epithelial cells with dense lymphoid stroma[2,3]. The various appellations have included lymphoepithelioma like carcinoma, undifferentiated carcinoma with lymphoid stroma, carcinoma ex lymphoepithelial lesion, and Eskimoma[3]. The incidence of LEC is 1.4% of the major salivary malignant neoplasms[4]. Previous studies showed a strong association between LEC and EBV among various ethnic groups including Eskimo, Chinese, Japanese, and Taiwanese[3-8]. However, some salivary LEC from Western patients were not associated with EBV[5]. The degree of association seemed to vary in different geographical regions. To the authors' knowledge, there has not been documented association in Thai patients. The present report describes three patients with EBV associated LEC of the right parotid gland. This appears to be the first report in Thailand and Southeast Asia. Clinical investigations, cytologic, histopathologic and immunopathologic features of parotid glands show typically characteristic findings of LEC.

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Case Report

Case 1

A 57-year-old Thai female patient living in Bangkok Thailand was admitted to Ramathibodi Hospital on February 2005, because of the painless, gradually enlargement of her right parotid gland of seven month's duration. She had a history of diabetes

mellitus, hypertension and dyslipidemia for four years' duration. She was placed on metformin, atenolol, amlodipine besylate, and simvastatin. There was no history of tuberculosis or malignancy among the members of the family. Physical examination of the right parotid gland revealed a firm, movable mass measuring 5 x 4 x 3 cm. There was neither facial palsy nor evidence of inflammation. The cervical lymph node could not be palpated. Fine needle aspiration (FNA) of the right parotid gland was performed and revealed intermingling of sparse atypical epithelial cells and some lymphocytes. A parotidectomy with excision of ten periparotid lymph nodes was performed.

The superficial lobe of the right parotid gland was firm, yellow tan and measured 5.5 x 4.5 x 3 cm. The cut surfaces showed partially circumscribed, indurate, white lesion measuring 3.8 x 3.7 x 2.4 cm that merged with the surrounding parenchyma (Fig. 1). The histopathologic feature revealed a diffuse growth pattern consisting of nests of tumor cells characterized by syncytial cytoplasm, large vesicular nuclei, prominent nucleoli and frequent mitoses, in a lymphoid stroma. Entrapped residual salivary acini and ducts were detected. Benign lymphoepithelial lesion was absent. Immunohistochemical stains showed tumor cells to be positive with cytokeratin (CK) (DAKO), and p53 (DAKO). Tumor cells were negative for leukocyte common antigen (LCA). Neoplastic cells in the periparotid lymph nodes were immunoreactive for CK and immunonegative for LCA. The malignant epithelial cells showed positive nuclear signals for EBV-encoded Ribonucleic acid (EBER) (DAKO) by ISH, whereas the lymphoid cells were negative. The deep lobe of the right parotid gland revealed sialosis. Computed topography (CT) of the nasopharynx was within normal limit. The pathological diagnosis of the right parotid tumor was LEC and classified as stage III (T3N1M0). The postoperative course was uneventful. Postoperative radiotherapy was administered. She was still uneventful until two months after the operation.

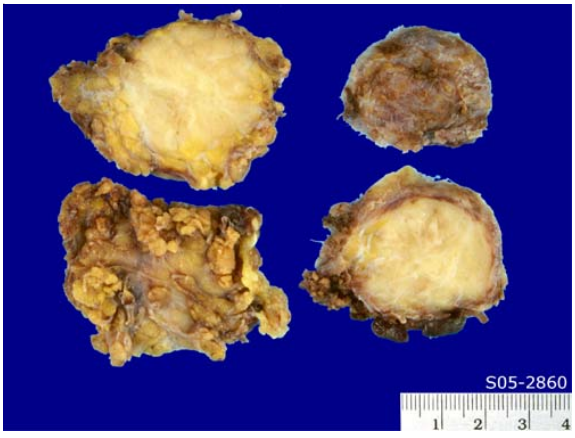

Fig. 1 The section of the right superficial parotid gland shows partially circumscribed, indurate, white lesion

Case 2

A 39-year-old Thai female farmer living in Roi Et Thailand, came to Ramathibodi Hospital on November 2004, complaining of a firm swelling mass of the right parotid gland of fourteen months' duration. The mass showed a slowly progressive lesion without tenderness. Physical examination of the right parotid gland revealed a mobile nodule measuring 3.5 cm in diameter. FNA of the right parotid gland was performed but was unsatisfactory for diagnosis. The patient underwent exploratory surgery and resection of the right superficial parotid gland was performed.

The surgical specimen consisted of a circumscribed homogenous tan mass measuring 3.5 x 2 x 1.5 cm. Histological analysis demonstrated tumor cells growing in nests and trabecular sheets, and arranged in large lobules (Fig. 2). Neoplastic cells exhibited syncytial appearance with a large vesicular nuclei and prominent nucleoli (Fig. 3). Tumor cells were closely intermingled with lymphoid cells. There was no epimyoepithelial island. The immunohistochemical stains for CK and p53 were positive in malignant epithelial cells. Tumor cells were negative for LCA. ISH showed the neoplastic cell was strongly and diffusely labeled by the EBER probe. Residual normal salivary acinar cells, lymphoid cells and histiocytes were negative. Multiple biopsies of the nasopharynx were negative for malignancy. Multiple biopsies of the nasopharynx were negative for malignancy. The pathological diagnosis of the right parotid tumor was LEC and classified as stage II (T2N0M0). The postoperative course was unevenful. She received radiotherapy with the total dose of 60 Gy. The patient is currently disease free at 5 months after surgery.

Case 3

A 24-year-old healthy Thai male living in Phetchaburi, Thailand, came to Ramathibodi Hospital on December 2003 with the complaint of progressive right parotid gland swelling, starting 6 months prior to admission. The patient had no history of significant illness in the past. The physical examination revealed a well defined, firm mass measuring 2 cm in diameter. There was neither facial palsy nor evidence of inflammation.

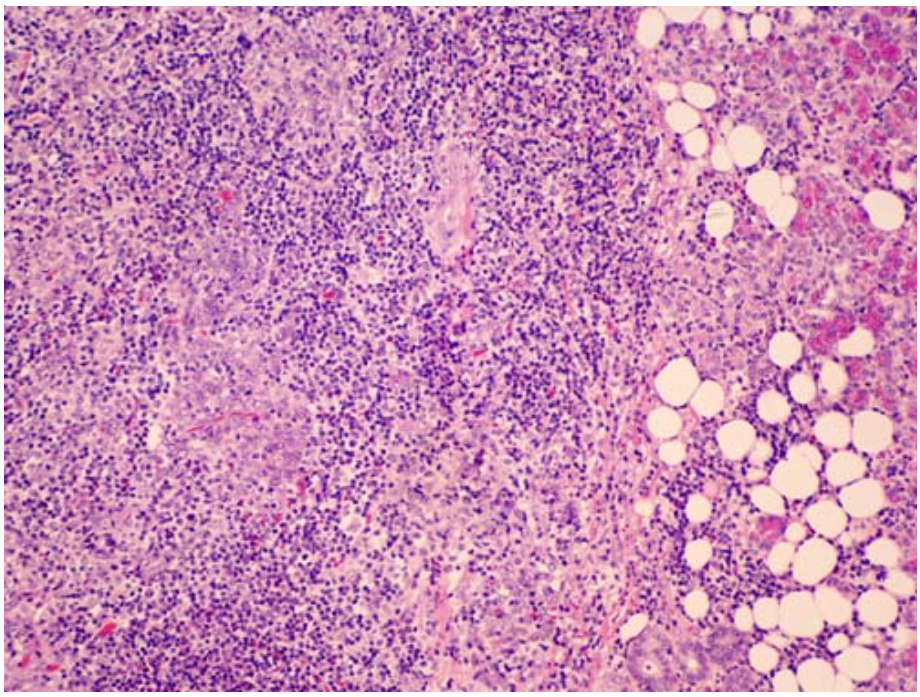

Fig. 2 The histopathologic feature of the right parotid gland reveals tumor cells growing in nests and trabecular sheets, and arranged in large lobules. H&E, X100

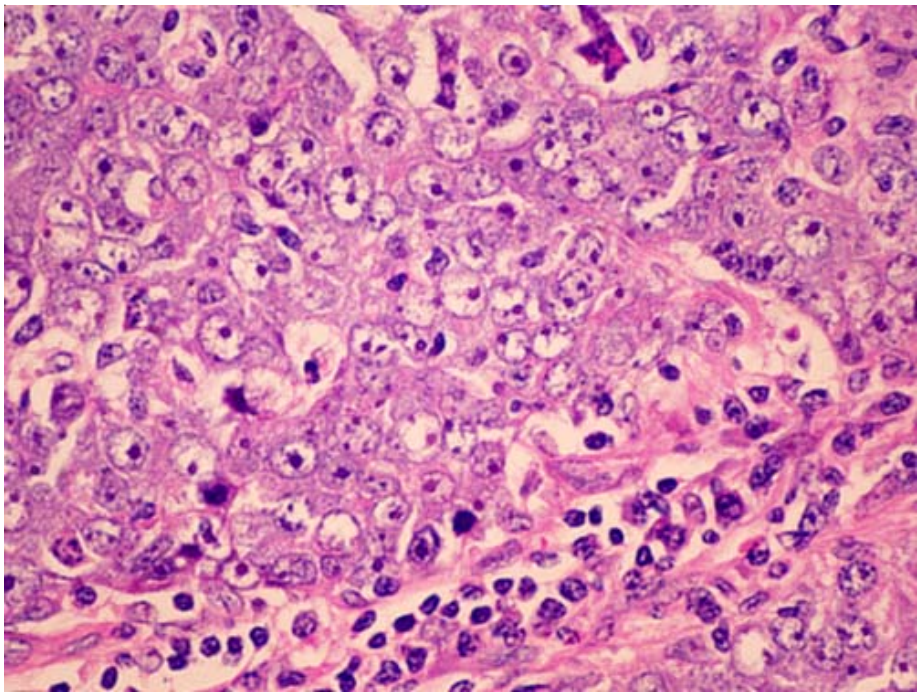

Fig. 3 The histopathologic feature of the right parotid gland reveals neoplastic epithelial cells exhibiting syncytial appearance with a large vesicular nuclei and prominent nucleoli. H&E, X400

The cervical lymph node could not be palpated. An evaluation of nasopharynx and lungs were within normal limit. FNA of the right parotid gland was performed and revealed chronic sialadenitis. Excision of the right superficial parotid gland was performed.

The resected gland contained a white and firm mass with a lobulated cut surface, which measured 2.5 x 2.3 x 2.2 cm. Histopathology of the tumor showed a malignant lymphoepithelial lesion characterized by a nest of carcinoma with abundant lymphoid stroma. The tumor nests consisted of syncytial sheets composed of polygonal cells with large, pleomorphic and vesicular nuclei with prominent eosinophilic nucleoli. The lymphoid stroma was composed of a polymorphic mixture of T and B-lymphocytes and a few plasma cells. No area of benign lymphoepithelial lesion was detected. The immunohistochemical stains for CK and p53 were positive in malignant epithelial cells. Tumor cells were negative for LCA. ISH for detection of EBV by using EBER probe disclosed abundant EBER in malignant epithelial cells, but not in the surrounding lymphoid stroma (Fig. 4). The pathological diagnosis of the right parotid tumor was classified as stage II (T2N0M0). The postoperative course was uneventful. The patient refused radiotherapy. He was free from recurrence 16 months after superficial parotidectomy.

Discussion

LEC is a relatively uncommon primary malignant tumor of the salivary gland with striking geographic and ethnic distribution[2,3]. Greenland Eskimos and Asian Orientals have the greatest incidence of LEC[2-8]. The average age at presentation occurs principally during the fourth to fifth decade[3]. The reported ages of patients range from 10 to 86 years[3]. This tumor shows a female predilection of approximately 3:2[3]. The most frequently presenting symptoms of LEC are salivary gland swelling, often associated with pain or discomfort and seldom associated with a seventh cranial nerve palsy[3,9]. Many patients have cervical lymphadenopathy at the time of diagnosis[3,9]. The routine initial laboratory investigations are noncontributory. Fine needle aspiration may allow early recognition of malignant salivary gland tumors. The most common site of metastasis is regional lymph node (41.3%)[9]. Distant metastases usually involve the lung, liver, and bone[3,6,8].

The macroscopic findings of LEC have been variably described as encapsulated, partially circumscribed, multinodular and infiltrative[3,9,10]. Some tumor

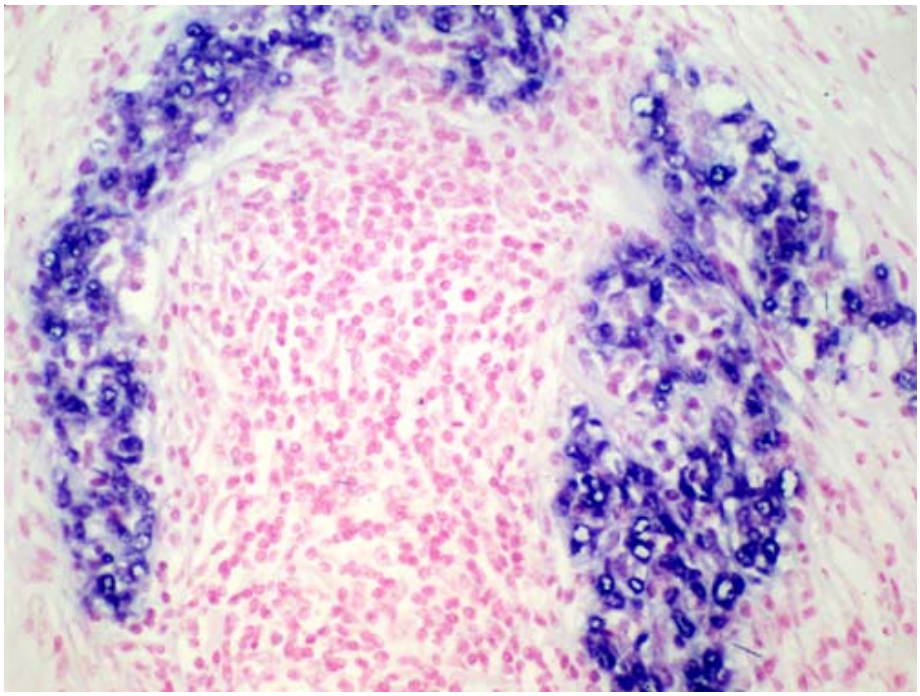

Fig. 4 In situ hybridization for detection of EBV by using EBER probe discloses abundant EBER in malignant epithelial cells, but not in the surrounding lymphoid stroma. X200

invades the extrasalivary parenchymal tissue[3]. The tumor size ranges from 1 to 10 cm[3]. The microscopic findings demonstrate circumscribed or irregular infiltrates of densely aggregated lymphoid cells with irregular shaped islands of eosinophilic epithelioid cells[2-10]. The architectural growth patterns of these epithelioid cells are small islands, syncytial masses, cords, trabeculae, or isolated cells. Epithelial islands are frequently widely separated by the lymphoid-rich stroma. The malignant epithelial cells have abundant amphophilic to eosinophilic cytoplasm with large, round to oval, lightly basophilic to vesicular nuclei that usually contain one or more prominent nucleoli[2-10].

The differential diagnosis of primary malignant neoplasms of the salivary gland includes lymphoma, and metastatic undifferentiated carcinoma[2, 3]. Lymphoma histologically shows atypical lymphocytes infiltrating replace acini and ducts, surround the nerve, and spill into fat and interlobular and periglandular connective tissue. Negative results of immunohistochemical stains for LCA may be helpful in excluding lymphoma. The metastatic undifferentiated carcinoma was histologically indistinguishable. In the presented cases, the metastases from nasopharyngeal lesion are excluded by normal results of CT of the nasopharynx, nasopharyngoscope and multiple random nasopharyngeal biopsies.

The pathogenesis of LEC is controversial. The relationship between the LEC and its benign counterpart has not been completely established. One third of the reported cases have implied that LEC arisen from epimyoepithelial islands of benign lymphoepithelial lesion[9]. On the other hand, two thirds of the published cases in which the malignant process appears to arise *de novo*[9]. There is a report of positive result of ISH for EBER and p53 immunostaining in malignant epithelial cell of LEC, but negative result in a benign lymphoepithelial lesion[7]. These findings suggest that LEC and benign lymphoepithelial lesion are not pathogenetically related. In the presented cases, the authors believe that the reported cases arise from metaplastic ductal epithelium and are not the consequence of evolution of benign lymphoepithelial lesions by the evidence of lack of benign lymphoepithelial lesion by histopathology, in combination with positive results of p53 immunohistochemistry and ISH for EBER in malignant epithelial cells.

Patients previously reported with LEC of the salivary gland from Greenland Eskimos and Asian Orientals had been identified EBV- Ribonucleic acid by ISH in the neoplastic cells[2-8]. Furthermore, there are few reports in the literature documenting the elevated titers of IgA antibodies to EBV capsid antigen[11]. It is suggested that EBV infection may be a factor in the development of these salivary neoplasms. EBV may be directly shed from lymphocytes into the salivary gland or may replicate in epithelial cells. However, the increased virus shedding, in combination with possible genetic changes such as the absence of *p16INK4a* expression[12], results in establishment of a latent infection in a basal epithelial cell. The viral episome is maintained in the infected epithelial cell that continues to proliferate and does not differentiate. This focus of latent infected, metaplastic epithelial cells rapidly become malignant and invades the basement membrane. However, some salivary LEC from Western patients were not associated with EBV[5]. The presented cases are the EBV associated with LEC in the three Thai patients. The present findings suggest that predisposing genetic factors may not play a major role in susceptibility to EBV infection. Since study of the relationship between EBV and LEC is still scarce, the role of EBV in the pathogenesis of salivary LEC should be further investigated.

Wide surgical excision remains the cornerstone of surgical management of salivary gland tumors. Some literatures suggest combined surgery, including neck dissection and radiotherapy, in either a primary or adjuvant setting to the tumor bed and ipsilateral neck[3,13]. However, the role of chemotherapy therefore awaits further investigation. The 5-year survival rate for all stages and grades of LEC is 66%[13]. The prognosis of LEC seems to be better than other types of undifferentiated carcinoma[3,4,6,9,13]. This raises the possibility of the lymphocytes having a direct protective function by destroying the malignant cells, resulting in a better prognosis of this subtype of undifferentiated carcinoma of the salivary gland. However, the presence or absence of EBV in LEC does not appear to be of prognostic significance[3,4].

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รายงานผู้ป่วยมะเร็งที่ต่อมน้ำลายข้างหูชนิดลิมโฟเอพิธเลียลพบร่วมกับการติดเชื้อไวรัสอีบีวี

นพดล ลาภเจริญทรัพย์, ณัฐา ทับทอง, วีระพล ประณีตวตกุล, อัจฉราพร พงษ์ทิพพันธ์, ยุวดี เลี่ยวไพรัตน์, วรชัย ศิริกุลชยานนท์

คณะผู้เรียบเรียงรายงานผู้ป่วยมะเร็งปฐมภูมิที่ต่อมน้ำลายข้างหูชนิดลิมโฟเอพิธเลียล พบร่วมกับการติดเชื้อไวรัสอีบีวี ในผู้ป่วย 3 ราย มาพบแพทย์ด้วยอาการก้อนทูมที่ต่อมน้ำลายข้างหูขวา ได้ทำการผ่าตัดและตรวจทางพยาธิวิทยาพบเป็น กลุ่มเซลล์มะเร็งเอพิธเลียล ล้อมรอบด้วยเซลล์เม็ดเลือดขาว ซึ่งเซลล์มะเร็งเอพิธเลียล มีการแสดงออกของแอนติเจนเซลล์เยื่อบุผิว และ p53 ตรวจทางอิมมูโนวิทยา ด้วยเทคนิค In situ hybridization พบ RNA จีโนมของไวรัสอีบีวี ซึ่งเป็นรายงานกรณีศึกษาแรกของประเทศไทยและเอเชียตะวันออกเฉียงใต้ พร้อมกันนี้ได้ทบทวนจดหมายเหตุการแพทย์ โดยรวบรวมวิเคราะห์การแสดงออกทางคลินิกและพยาธิวิทยา